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ALLELOPATHIC POTENTIAL OF *JATROPHA CURCAS* L. LEAF AQUEOUS EXTRACTS ON SEEDLING GROWTH OF WHEAT

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Abstract

Allelopathic effects of aqueous leaf extracts of *Jatropha curcas* on seed germination and early seedling growth of wheat cv. Inqlab-91 were investigated. The extracts were applied at 50%, 25%, 12.5%, 6.25% and 3.12% as seed soaking for 5h prior to sowing of seeds in the pots. The *J. curcas* leaf characterized for composition of macronutrients showed Na (304 µg/g), K (267 µg/g), Mg (92 µg/g) and Ca (12 µg/g). Among micronutrients Fe (92 µg/g), Cr (92 µg/g), Ni (48 µg/g), Co (38 µg/g), Cu (23 µg/g, Mn (12 µg/g) and Zn (15.22 µg/g) were found. Phenolic compounds were detected in the extracts and were found maximum (8.12 mg gallic acid/g extract) in 50% extract. Lower concentrations (6.25%, 3.25%) of the extracts significantly improved seed germination (%), germination index, shoot length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root area of wheat plants ($p<0.05$). At higher concentration of the extract, root length was significantly reduced. It is inferred that lower concentrations (6.25% and 3.12%) of the extracts exhibited beneficial effects on growth of wheat plants.

Key words: Allelopathy, Phenolics, *Jatropha curcas*, Nutrients, Germination.

Introduction

The process by which a plant species inhibits or stimulates the growth and development of other neighboring plant species through the production of secondary metabolites is called allelopathy (Levin, 1976; Barkatullah *et al.,* 2015). In developed countries such as USA, Korea, Canada, Russia, Japan, Mexico and Australia allelopathy provides source to sustainable agriculture. Allelopathy is not restricted to a single field rather to multidisciplinary areas of research including soil science, agronomy, plant breeding, genetics, agro forestry and vegetable crops. It is concerned with plant protection i.e. insects control, nematodes control and diseases control. Allelopathy also deals with the problems of weeds i.e. crop obstruction (Bell & Koeppe, 1972) and phytotoxcity in stubble mulch farming (McCalla & Haskins, 1964). Several investigators have worked on occurrence of allelochemicals in plants and microorganisms. These allelochemicals were present in the upper parts i.e. stem, leaves and flowers or in underground parts of plant i.e., roots or in both parts i.e. upper and underground parts (Rice, 1974).

A number of allelochemicals are present in cells and tissues of plants (Ashrafi *et al.,* 2008; Gilani *et al.,* 2007). Recent researches have shown that phenols were the most effective substances on germination, seedling growth and cell division (Khan *et al*., 2011). Allelochemicals have a variety of chemical structures and actions. However, their effects on biochemical and physiological processes of target plants have not been investigated thoroughly (Hussain *et al*., 2010). Current studies showed that higher phenolic contents were negatively correlated with seed germination (%) and growth indices of canola (Ullah *et al.,* 2014).

Jatropha curcas has gained popularity as biodiesel plant in both developed as well as developing countries of the world. Its seeds contain predominantly crude fat (oil), protein and fibers (Brittaine & Lutaladio, 2010). Jatropha oil is non-edible and is mainly used as biodiesel energy (Achten *et al.,* 2009; Ullah *et al*., 2014). In Pakistan cultivation of *J. curcas* is expanding rapidly because of government interest in renewable energy. Therefore it is necessary to evaluate phytotoxic effect of *J. curcas* on agricultural crops before its introduction into the agro forestry system. Allelopathic impacts of aqueous extracts of *J. curcas* on seed germination and growth of wheat are investigated in the present study.

Materials and Methods

Fresh leaves of *J. curcas* were collected; air dried at room temperature and ground finely using an electric grinder. The fine powder of leaves (600 g) was soaked in 1L of distilled water at room temperature for 48 h. Whatman No. 1 filter paper was used for filtration of the aqueous extract. The stock solution thus prepared was further diluted to make 50%, 25%, 12.5%, 6.25% and 3.125% solutions.

Elemental analysis of *J. curcas* leaf: The leaf powder was analysed for both macronutrients (Na, K, Ca and Mg) and micronutrients (Mn, Fe, Zn, Cr, Co, Ni and Cu) using standard protocols (Rashid, 1986; Ryan *et al*., 2001). Powdered leaf material was taken in a conical flask (50 ml) having 10 ml mixture of Nitric-Perchloric acid (2:1). The temperature of the flasks was raised upto 150^{o}C until color of the fumes became white. After cooling, extract was filtered at room temperature. The volume of filtrate was increased to 50 mL with addition of DH_2O. Atomic Absorption Spectrophotometer (Perkin Elmer Analyst-200, USA) was used for elemental analysis.

Total phenolic compounds analysis of aqueous extracts: Folin-Ciocalteau method was used for determination of total phenolics (Wolfe *et al.,* 2003). Extract (200µl) was mixed with Folin Ciocalteu reagent (diluted and freshly prepared) and 7.5% Na_2CO_3 (2 ml).

The mixture was further diluted with distilled water to a final volume of 7 ml and was kept in dark for 2 h. Measurements of the absorbance were made at 765nm by using a spectrophotometer (Hitachi's U-510 Japan). Standard curve was generated by using various concentrations of gallic acid and the measurements were compared to the plotted standard curve. The content of total phenolics was articulated as mg gallic acid equivalents / g sample.

Plant material and growing conditions: Pot experiment was performed in the glass house of Department of Botany University of Science and Technology, Bannu KP Pakistan. Seeds of wheat cv. Inqlab-91 were collected from Agriculture Research Station Bannu and surface sterilized with 0.2% solution of mercuric chloride for 2-3 mints, subsequently washed 3-4 times with autoclaved distilled water. Seeds were soaked in various concentrations of the extract for 5h provided with proper aeration. The following treatments were made.

Treatments	Concentrations used
Control (T0)	Plants supplied with autoclaved distilled water
T1	Plants supplied with 50% extract solution
T2	Plants supplied with 25% extract solution
T3	Plants supplied with 12.5%extract solution
T4	Plants supplied with 6.25% extract solution
T5	Plants supplied with 3.12% extract solution

Sterilized grains of wheat were sown in plastic pots measuring 11x8 cm^2 filled with sand and clay (1:1). The experiment was arranged in complete randomized design (CRD).

The soil used as culture medium for growing of wheat plants was analyzed for pH, nitrogen and available phosphorous (P). The method of Mclean (1982) was used for the determination of soil pH. Method of Olsen & Sommers (1982) was used for the estimation of phosphorous (P) content in the soil. For determination of soil nitrogen content, 10 g soil sample was digested in micro-kjeldahl flask having concentrated H_2SO_4 (20 ml) and digestion mixture (10 g). After cooling, the contents of the micro-kjeldahl flask were transferred to a conical flask (50 ml). The distillation of the content was done with 40 % NaOH (20 ml) and was collected in HBO_3 (5 ml). Titration of the distillate was carried out against H_2SO_4 (0.1 N) using potassium permanganate ($KMNO_4$) as indicator.

Germinated seeds in all treatments were counted on daily basis for seven consecutive days. At 8^{th} day of the experiment, the seedlings were harvested for further analysis.

Seed germination percentage (%): The determination of seed germination (%) was made:

$$\text{Seed germination (\%)} = \frac{\text{Germinated seeds}}{\text{Total number of seeds grown}} \times 100$$

Germination index: Germination index (GI) was determined by standard formula as given by Association of Official seed Analysts (Anon., 1983).

Germination index (GI) = Seeds which were germinated at first count + Number of seeds which were germinated at last count / Days of first count + days of final count

Germination rate index: The germination rate index (GRI) was calculated as follow:

$$\text{GRI} = \frac{\text{Germination index}}{\text{germination percentage}}$$

Mean germination time (MGT): For calculation of mean germination time equation of Ellis and Roberts (1981) was used.

$$\text{MGT} = Dn/n$$

where: n represents the total number of seeds having germination on day D, D represents the total number of days counted from the beginning of germination.

After harvest, measurement of shoot length and root length was taken by a common measuring tape. Shoot fresh weight and shoot dry weight, root fresh weight and root dry weight were measured using electronic balance. Root area was determined using Root Law Software (Washington State Research Foundation USA).

Statistical analyses: The data was analysed using one way ANOVA. Means values of treatments were compared using least significant differences (LSD) test (Steel & Torrie, 1984). Student Statistix (version 8.1 USA) was used for determination of the coefficient of correlation.

Results and Discussion

Soil filled in pots had a pH value of 6.90, total nitrogen 0.036 (%) and available phosphorous 4.38 µg/g DW. The *J. curcas* leaf characterized for composition of macronutrients showed Na (304 µg/g), K (267 µg/g), Mg (92 µg/g) and Ca (12 µg/g). Among micronutrients Fe (92 µg/g), Cr (92 µg/g), Ni (48 µg/g), Co (38 µg/g), Cu (23 µg/g, Mn (12 µg/g) and Zn (15.22 µg/g) were detected (Fig. 1).

Maximum phenolics content (8.12 mg gallic acid /g extract) was recorded in 50% extract followed by 25% (7.85mg gallic acid /g extract), 12.5% (7.4 mg gallic acid /g extract), 6.25% (6.88mg gallic acid /g extract) and 3.12% (4.5 mg gallic acid /g extract) extract respectively (Fig. 2).

Allelochemicales are secondary metabolites produced by plants and their effect may be stimulatory or inhibitory on growth and development of neighboring plants (Levin, 1976). Previous studies have shown that majority of the compounds having hazardous effects on neighboring plants are phenolics in nature (Khan *et al*., 2011; Ullah *et al*., 2014). During present investigation, phenolic compounds were detected in aqueous extract of *J. curcas*. These results confirmed findings of Sharma *et al.* (2009) who have reported that aqueous extracts of plants contain phenolic compounds.

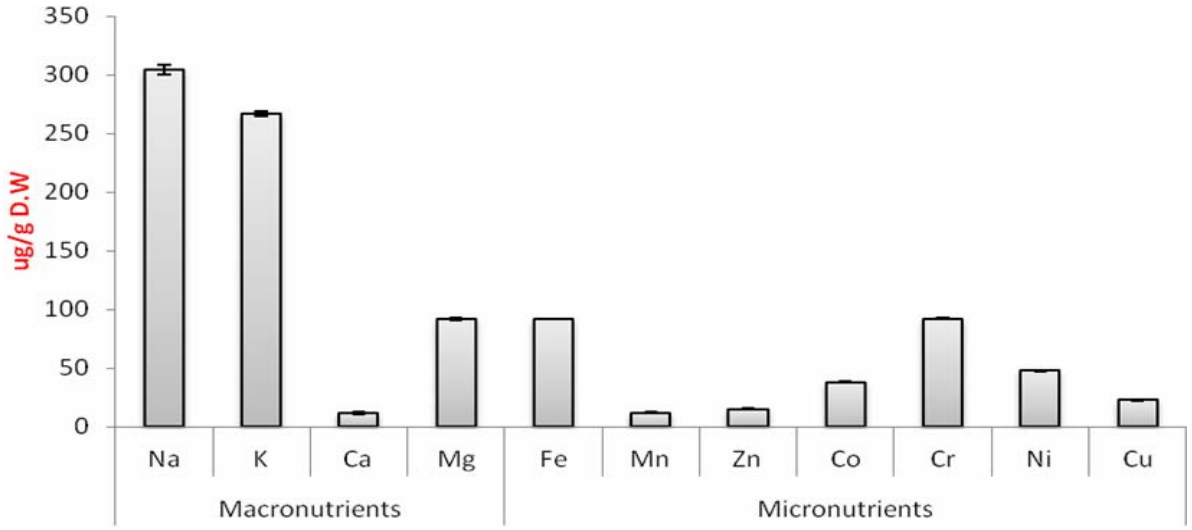

Fig. 1. Elemental composition of *J. curcas* leaf.

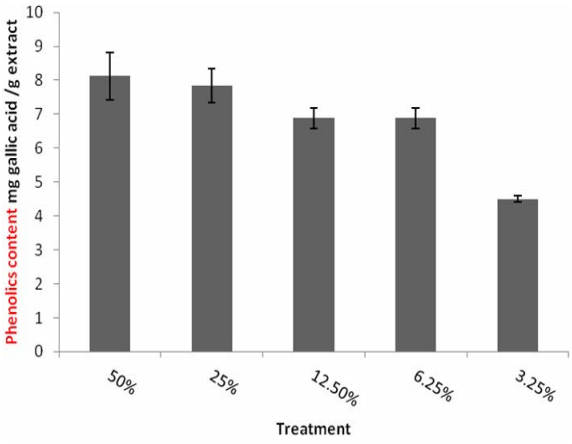

Fig. 2. Phenolic contents of aqueous extracts of *Jatropha curcas* leaves.

Extracts of *J. curcas* were either ineffective or stimulatory for seed germination (%) and germination index of wheat. It was observed that stimulatory effect was greatest at lower concentrations of the extract as compared with control. Effects of extract on germination rate index and mean germination time were non-significant (Fig. 3a, b, c, d).

The findings of the current investigation were similar to those of Tomar & Agarwal (2013) who have reported effects of leachates of sun dried leaves and ovary walls of *J.curcas* on wheat. They found non-significant impacts of leachates on seed germination and growth of wheat plants. The results of a study conducted by Nadaletti *et al*. (2014) showed a significant and positive effect of *J. curcas* aqueous extracts on seedling growth of cauliflower.

There was positive and significant correlation of total phenolics content of extracts with germination (%) (r=0.715) and germination index (r=0.592). The germination (%) was positively significantly correlated with germination index (r=0.9358).

Extracts at all concentrations have no affect on shoot length and leaf area of wheat plants as compared with control. At lower concentration (6.12% and 3.25%) significant increase was recorded in fresh weight and dry weight of shoot over control (Fig. 4a, b, c, d).

Aqueous extracts at all concentrations significantly reduced root length of wheat plants than control. However, at lower concentrations of the extract, there was significant increase in root area, root fresh weight and root dry weight as compared with control ($p<0.05$) (Fig. 5a, b, c, d).

The correlation of root fresh weight and root dry weight was positive and significant with total phenolics content of extracts (r=0.732, r=0.492). The correlation of root fresh weight was positive and highly significant with root dry weight (r=0.884).

The inhibition in root length of target plant species in response to allelochemicals is a good indicator of phytotoxicty. Previous studies have shown significant decreases in root length at higher concentrations of the plant extracts (Rafiqul Hoque *et al*., 2003; Amoo *et al*., 2008). Root area determines the region where active absorption of nutrients and water takes place (Jackson *et al*., 1997). The advantageous effects of *J.curcas* aqueous extracts on wheat plants were related to exceptional changes in root surface area. The beneficial effects of extract on fresh weight and dry weight of wheat plants indicated that *J. curcas* extracts resulted in accumulation of dry matter. This might be because those *J. curcas* leaves have certain allelochemicals and nutrients which can promote the growth of wheat plants. Previous studies have shown that allelochemicals can be either stimulatory or inhibitory (Lawan *et al*., 2011). In the present investigation, allelochemicals present in aqueous extracts of *J. curcas* were found stimulatory to wheat at lower concentrations. At higher concentration the extracts were either inhibitory or did not affect the growth of wheat plants. This might be because that biological activity of allelochemicals is concentration dependent (Einhellig, 1986). There is a need to assess the allelopathic compatibility of wheat with *J. curcas* under long term field conditions since natural conditions such as physicochemical properties of the soil and the activity of microbes in rhizosphere region could lessen or increase this effect (Kamara *et al*., 1999).

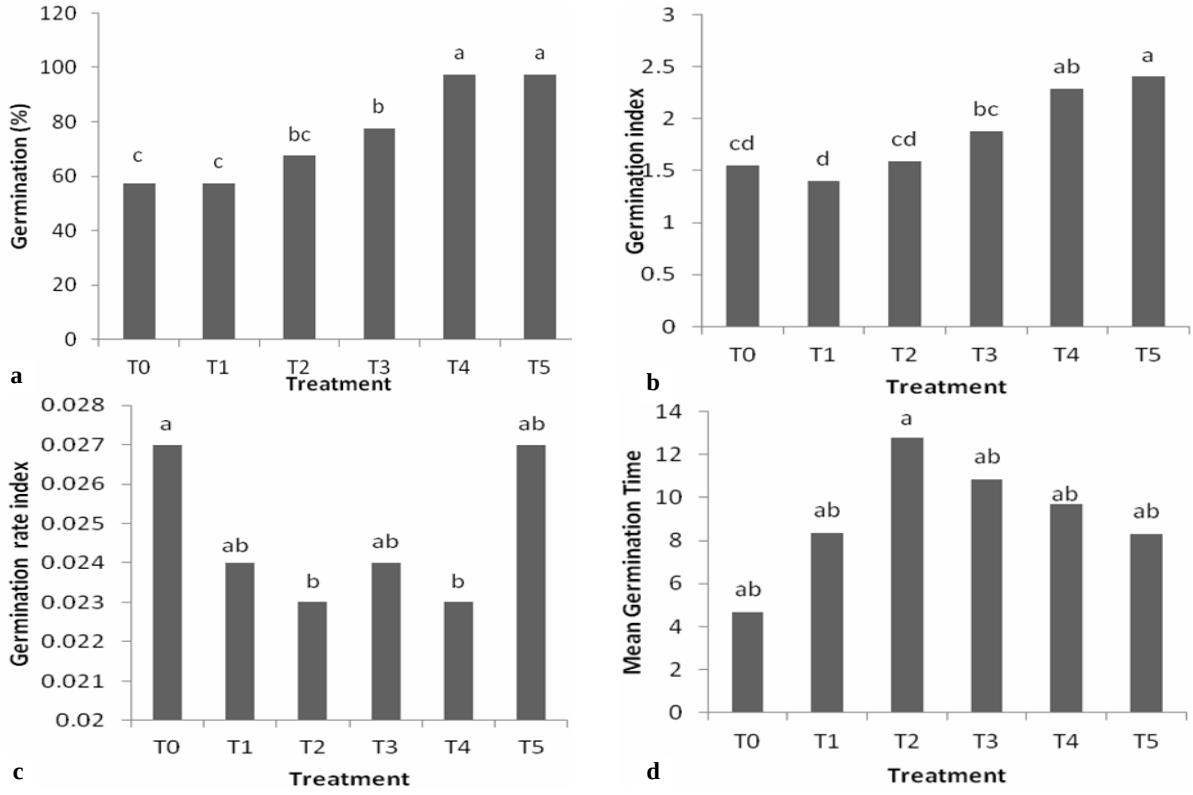

Fig. 3. Effects of aqueous extracts of *Jatropha curcas* leaves on (a) Germination (%), (b) Germination index, (c) germination rate index, (d) mean germination time of wheat.

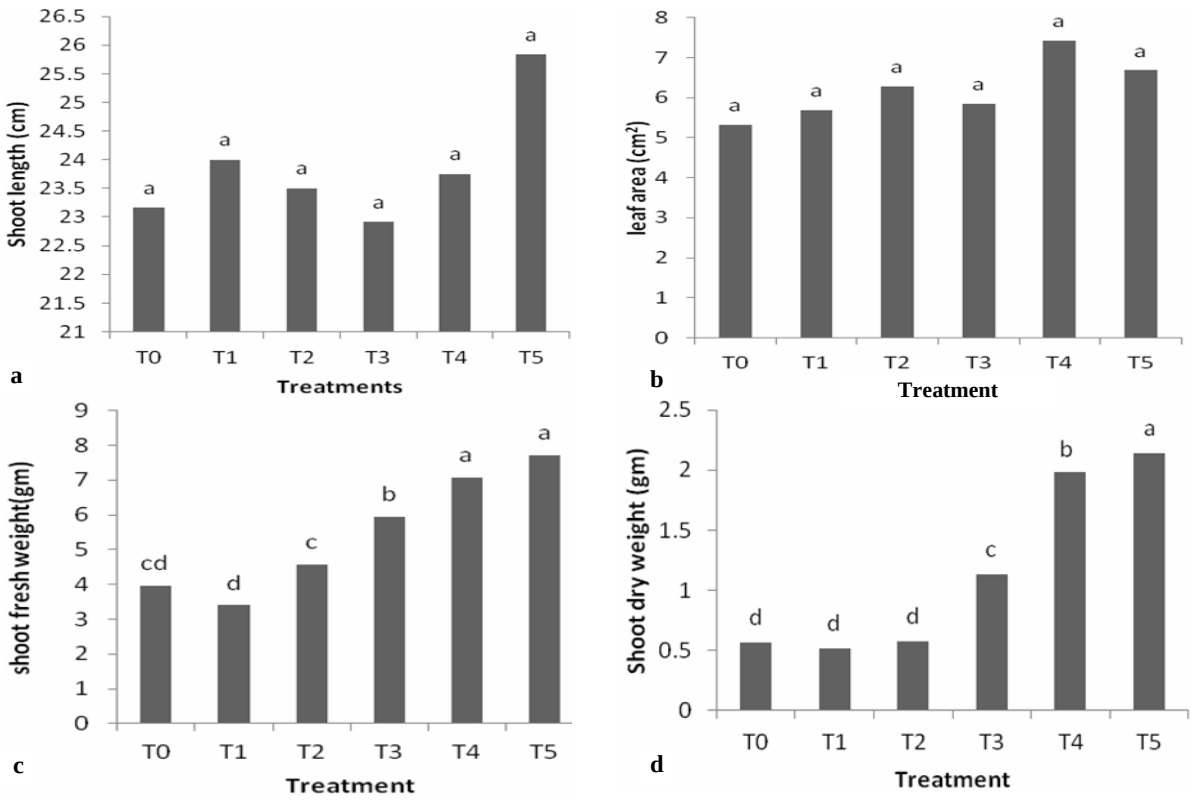

Fig. 4. Effects of aqueous extracts of *Jatropha curcas* leaves on (a) shoot length, (b) leaf area, (c) shoot fresh weight, (d) shoot dry weight of wheat.

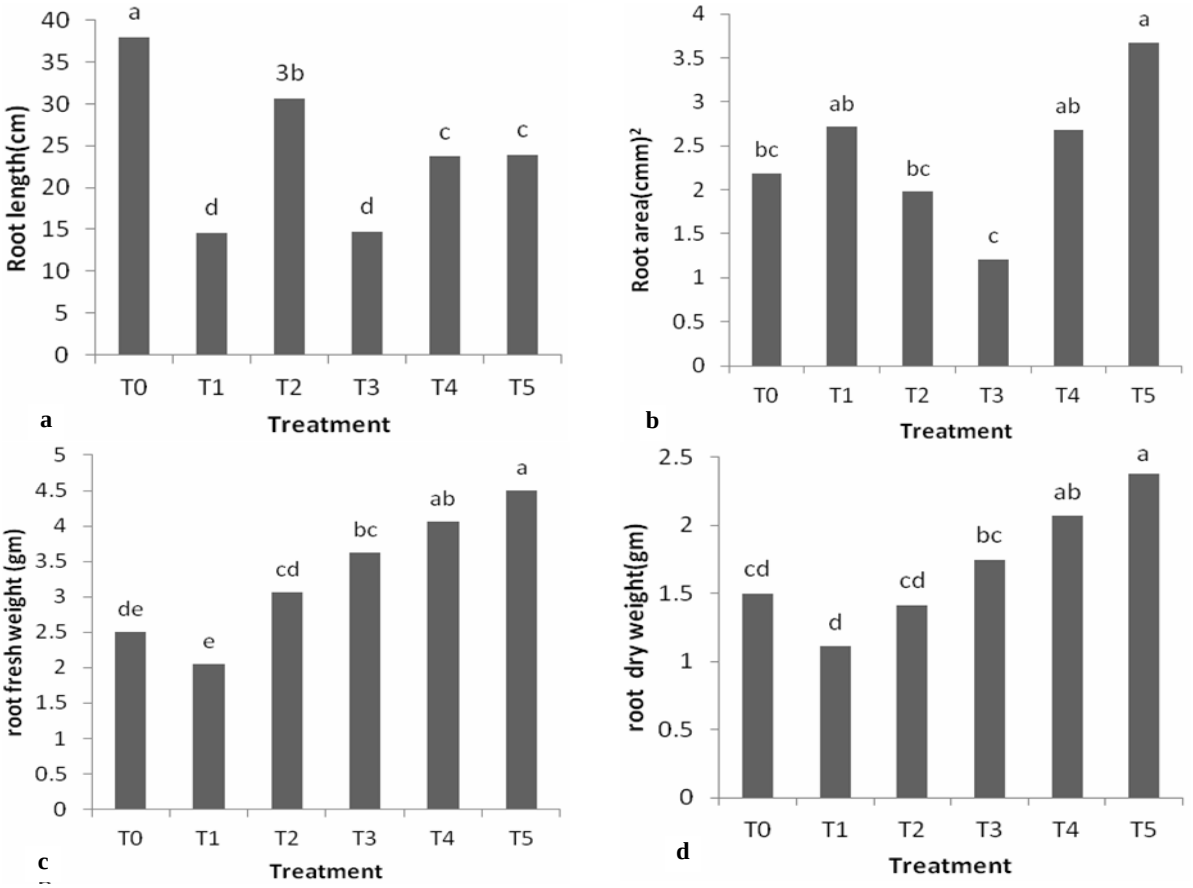

Fig. 5. Effects of aqueous extracts of *Jatropha curcas* leaves on (a) root length, (b)) root area, (c) root fresh weight, (d) root dry weight of wheat.

Conclusion

The leaf of *J. curcas* was a good source of macronutrients and micronutrients. At lower concentrations, phenolics present in aqueous extracts of *J. curcas* stimulated seed germination and growth indices of wheat. Findings of present investigation concluded that lower concentrations of *J. curcas* leaf aqueous extract were stimulatory on growth of wheat and could lead to the formulation of some novel, sustainable, environment friendly and economical bioregulator.

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